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## GENERATION OF HIGHLY DIVERSE LIBRARY OF EXPRESSION VECTORS VIA HOMOLOGOUS RECOMBINATION IN YEAST

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### ABSTRACT

Methods are provided for generating highly diverse libraries of  
10 expression vectors encoding fusion proteins such as single-chain antibodies  
via homologous recombination in yeast. The method comprises: transforming  
into yeast cells a linearized yeast expression vector having a 5'- and 3'-  
terminus sequence at the site of linearization and a library of insert nucleotide  
sequences that are linear and double-stranded; and having homologous  
15 recombination occur between the vector and the insert sequence such that  
the insert sequence is included in the vector in the transformed yeast cells.  
The insert sequence comprises a first nucleotide sequence encoding a first  
polypeptide subunit, a second nucleotide sequence encoding a second  
polypeptide subunit, a linker sequence encoding a linker peptide that links the  
20 first and second polypeptide subunits, and a 5'- and 3'- flanking sequence at  
the ends of the insert sequence which are sufficiently homologous to the 5'-  
and 3'-terminus sequences of the linearized yeast expression vector,  
respectively, to enable homologous recombination to occur. The first  
polypeptide subunit, the second polypeptide subunit, and the linker  
25 polypeptide are expressed as a single fusion protein; and the first and second  
nucleotide sequences each independently varies within the library of  
expression vectors.

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